

Absence of 6-Methoxybenzoxazolinone in Uninjured Maize Tissue

Sir:

The peculiar phenomenon has been demonstrated (1) that many living plants contain stable precursors which after the crushing of plants are enzymically decomposed to substances which (or after further chemical or enzymic decomposition) have different biological activities. One of the typical cases is the formation of benzoxazolinone (BOA) and its 6-methoxy derivative (MBOA) in crushed rye and maize plants, respectively. When these compounds were isolated and characterized chemically in this laboratory, it was at first believed that they were present in the intact plants (2). Later on we could relate their formation to the precursors present in these plants. The reaction series is: precursor, glucoside of 2,4-dihydroxy-7-methoxy-
enzymic
1, 4-benzoxazin-3-one \longrightarrow aglucone \longrightarrow

6-methoxybenzoxazolinone (3, 4). The last step in the reaction is of an unusual type—a six-membered ring is transformed into a five-membered one by the splitting of formic acid. When hydrolysis of the glucoside is completely prevented in the intact plant, we have never detected any formation of BOA or MBOA.

Smisman, *et al.* (5), recently reported that they found MBOA as an original substance in maize tissue. Quantitative data were not given. Their procedure was complicated, however, and involved some steps during which the formation of MBOA from the aglucone can take place. If the aglucone was already present in the ground plants when they were placed in 95% ethanol, the formation of some MBOA would be expected. Kinetic studies in this laboratory have shown that aglucone of the rye glucoside is converted into BOA at a much higher rate in 80% ethanol than in aqueous solution (6). The rapid conversion of the aglucone of the maize glucoside into MBOA in 95% ethanol solution at 40° is shown in Fig. 1.

The other step at which MBOA could surely be formed from the maize aglucone is the fractiona-

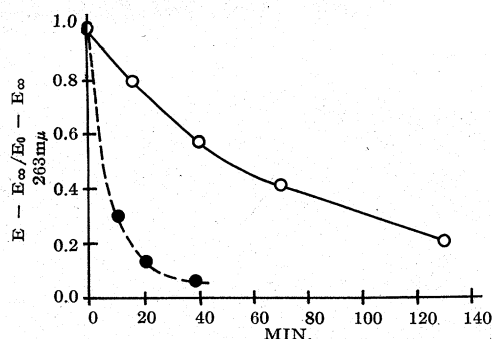


Fig. 1.—Decomposition of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one to 6-methoxybenzoxazolinone in 95% ethanol (solid line) at 40°C. and in 20% ethanol in ether in the presence of Woelm's acid aluminium oxide at 20°C. (broken line).

tion of the ethanol solution after ether extraction on an aluminium oxide (Merck) column. We have observed very rapid decomposition of the aglucone in the presence of this oxide (Woelm's preparation). The result is seen in Fig. 1. If the aglucone was present in the ether solution, MBOA was formed in the experiment of Smissman, *et al.* (5).

A simple and reliable method of inactivating the glucosidase and completely preventing the enzymic hydrolysis of the glucoside is to place the intact plant in ethanol solution (final ethanol concentration 70 to 80%) either at 0° or at a lower temperature for 24 hours. The temperature of the mixture is then allowed to rise to room temperature, after which the mixture is ground in a Waring Blendor and the alcohol evaporated in a vacuum. We made some new estimations of MBOA by immersing intact maize plants (Early Albert and a sugar maize variety) in a carbon dioxide-ethanol mixture (−76°) for 24 hours. The dry residue was extracted with ether, and the ether solution evaporated at about 0°. The residue was dissolved in a small volume of acetone and the solution mixed with a threefold volume of 2% acetic acid in water. The mixture was poured onto a small cellulose powder column and eluted with 2% acetic acid until 70 ml. of eluate was collected. The eluate was freeze-dried, and the residue dissolved in acetone and chromatographed on Whatman No. 1 paper with 2% aqueous solution of acetic acid as solvent. The aglucone has the R_f value 0.74 and MBOA 0.49 (ascending). The spots were

detected by U. V. absorption, the aglucone spot also by the formation of the blue-colored complex on spraying with alcoholic ferric chloride solution. Using these methods, we found neither the aglucone nor MBOA in young maize plants (about 2 weeks old). In older plants (1 to 2 months old) grown in the laboratory greenhouse under artificial light, when the tips of many of the leaves were already yellow, small amounts of aglucone (20 mcg./Gm. fresh wt.) were detected (about the amount of aglucone after enzymic hydrolysis see below), but no MBOA. It is possible, but not proved, that the aglucone was formed in the withered parts of the leaves, or that it is generally present in older plants.

When the enzymic hydrolysis of the glucoside was needed for the estimation of the aglucone and glucoside, the plants were ground in a mortar at about 10°. After standing for 1 hour the mixture was evaporated at low temperature and the dry residue extracted with ether and subsequently treated as described above. Five-hundred micrograms of aglucone was found per Gm. fresh wt. in 2-week-old maize plants (Early Albert), and 100 mcg. of aglucone per Gm. fresh wt. in 2-month-old plants. In 1-month-old sugar maize, the amount of aglucone was 200 mcg. per Gm. fresh wt. No MBOA could be detected in these plants either.

On the basis of our previous and present findings, we still consider that uninjured maize plants do not contain MBOA. The presence of free aglucone in small amounts in older maize plants is possible but requires additional evidence.

- (1) Virtanen, A. I., *Arch. Biochem. Biophys.*, **1**, 200(1962).
- (2) Virtanen, A. I., Hietala, P. K., and Wahlroos, Ö., *ibid.*, **69**, 486(1957).
- (3) Virtanen, A. I., and Hietala, P. K., *Suomen Kemistilehti B*, **32**, 38, 138, 252(1959).
- (4) Wahlroos, Ö., and Virtanen, A. I., *Acta Chem. Scand.*, **13**, 1906(1959).
- (5) Smissman, E. E., Kristiansen, O., and Beck, S. D., *THIS JOURNAL*, **51**, 292(1962).
- (6) Bredenberg, J. B., Honkanen, E., and Virtanen, A. I., *Acta Chem. Scand.*, **16**, 135(1962).

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